55. (Five Times Amended) A recombinant DNA comprising an expression vector comprising a promoter, a first DNA fragment and a second DNA fragment, said first DNA fragment being a DNA fragment of any one of claims 6, 7 or 9, said second DNA fragment being a DNA fragment comprising a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide having a property of enhanced toluene monooxygenase activity,

wherein the first DNA fragment containing a toluene monooxygenase encoding region of 4.9 kb or less is functionally connected to the promoter to express an active toluene monooxygenase, and the second DNA fragment is functionally connected to the promoter to express a property to enhance the toluene monooxygenase activity.--

REMARKS

This application has been reviewed in light of the Office Action dated August 13, 2002. The outstanding Claims are 1-48 and 55. Claims 1, 3, 5, 6, 8, 9, 10, 13, 17, 19, 21, 27, 33, 34, 48 and 55 have been amended to define more clearly what Applicants regard as their invention. Favorable reconsideration is requested.

Claims 1 and 8 have been amended to resolve informalities. Claim 13 has been amended, in compliance with 37 CFR 1.75(c), to be in proper form. It is acknowledged that Claims 2, 15, and 16 are allowed.

The Examiner rejected Claims 3-5, 9-14, 17, 18, 21-48 and 55 under 35 U.S.C. § 112, first paragraph, on pages 3-7 of the outstanding Office Action. In particular, on pages 3-4, the Examiner rejected Claims 3 and 10 on the grounds that the claims are not limited by function.

Claims 3 and 10 have now been amended to include such a function in order to address this rejection.

The Examiner further rejected Claims 3 and 10 on the grounds that the "stringent conditions" are not defined. That rejection is respectfully traversed.

The phrase "under stringent (hybridization) conditions" is a well-known and well understood expression to those of ordinary skill in the art. The degree of hybridization or homology between the probe and the nucleic acid is determined by the stringency of the hybridization. This stringency is determined by certain conditions, such as salt concentration and temperature. These conditions are easily adjustable to increase the stringency (or homology) or decrease the stringency. The methods for adjusting variables, such as salt concentration and temperature, so that conditions are stringent, are well-known in the art. These methods are known to students and are taught in college courses. For example, instructions for adjusting these variables so that stringent conditions exist, are found in college texts and primers, such as the text titled "DNA - Basics of Structure and Analysis" found on a university web-site at http://www.ndsu.nodak.edu/instruct/mcclean/plsc731/dna/dna6.htm, a copy of which is attached. Clearly, not only is this expression conventionally and broadly used in this art and well understood, but one of ordinary skill in the art can easily adjust conditions in any hybridization situation so that the conditions are stringent. Accordingly, the term "under stringent conditions" is adequately defined.

In addition, on pages 4-5, the Examiner rejected Claims 13 and 55 on the grounds that the claims contained no structural limitations wherein an enzyme is defined by the function only. Applicants respectfully traverse this objection.

First, the instant specification gives specific examples (Example 5, pages 56-58) of

the combination of DNA fragments for enhanced toluene monooxygenase activity, with one DNA fragment expressing an active monooxygenase and the second DNA fragment expressing a property to enhance toluene monooxygenase activity. Accordingly, there is a sufficient written description in the specification for Claims 13 and 55.

Moreover, as set forth in Claim 13 and elaborated in Claim 55, each DNA fragment has a specific sub-function. The first DNA fragment expresses an active monooxygenase. Meanwhile, the second DNA fragment expresses a property to enhance toluene monooxygenase activity. Taken together, the combined DNA fragments express an enhanced toluene monooxygenase activity. Accordingly, the claims establish definite boundaries on what is covered.

Also, on pages 6-7, the Examiner rejected Claims 3-5 and 10 as allegedly not being enabled because the claims are directed to a DNA encoding a polypeptide of an unknown function. Claims 3-5 and 10 have been amended to include a function in order to address this objection.

The Examiner, on pages 7-9 of the outstanding action, also rejected Claims 9, 11-14, 17, 18, 22-48 and 55 on the grounds that the specification does not provide enablement for a DNA encoding a toluene monooxygenase and TomK of an unknown amino acid sequence encoded by a DNA that hybridizes to said sequences. Applicants respectfully traverse the ground objection.

First, in order to expedite prosecution, Applicants have omitted the term "mutant".

However, the term "or a complement thereof" remains since it is well-known in the art that each DNA sequence has two nucleotide strands that are unique complements of one another. Known techniques such as PCR demonstrate that one of ordinary skill in the art can easily determine or make the complement of any nucleotide sequence, and vice versa. Moreover, well-known hybridization techniques involve the base pairing of a probe with its complement as a detection method. Since a nucleotide sequence inherently has a unique complement, once one of

ordinary skill in the art is able to detect and/or make a specific nucleotide sequence, one can just as easily then detect and/or make its complement, and vice versa. Accordingly, there is no undue experimentation required since the specification provides enough support so that one of ordinary skill in the art would be able to make a DNA fragment encoding a toluene monooxygenase or TomK having the sequence that comprises SEQ ID No:1 and its 234-443 portion, or complement thereof.

The Examiner had rejected to Claims 3, 5 -14, 17-48 and 55 under 35 U.S.C. § 112, second paragraph, as being indefinite, on pages 9-11 of the outstanding action.

Specifically, on page 10, the Examiner rejects Claims 3, 9, 10, 13 and 17 on the grounds that the term "stringent hybridization conditions" renders the metes and bounds of the claims unascertainable. The objection is respectfully traversed.

As explained above, not only is the term "stringent hybridization conditions" well-known in the art, but the conditions required for stringent hybridization are well-known, in that one of ordinary skill in the art can readily adjust conditions for any hybridization so that they are stringent, resulting in a high homology between the probe and the nucleic acid.

The rejections of claims 5, 6, 9, 13, 17, 19, 21, 27, 33, 34, 48 and 55, and all claims dependent from these claims, advanced on pages 10-11 of the outstanding Office Action, have been resolved by appropriate amendment.

Finally, the Examiner rejected Claims 22-48 under 35 U.S.C. § 101 on the grounds that the claimed recitation provides for the use of the transformant, but does not set forth any steps involved in the method or process of its use. This rejection is respectfully traversed, since it is based on an improper reading of the claims.

Claim 22, for example, does not merely claim a use of a transformant, as otherwise stated in the rejection. Claim 22 claims a "method for degrading" certain aliphatic or aromatic

compounds, involving the step of using a particular transformant. Such a claim is far different from the mere "use" claims that characterized the Examiner's cited cases of Dunki and Clinical Products. One claim in Clinical Products, for example, reads as follows:

> "The use as a sustained release therapeutic agent in the body of ephedrine absorbed upon polystyrene sulphonic acid."

Such a claim is devoid of any steps whatsoever, and is clearly a pure "use" claim. Claim 22, on the other hand, includes the step of "using" which is applied in the method of degrading compounds; such distinctions make the rejected claim for more than a pure "use" claim.

Applicants have endeavored to meet each of the points raised by the Examiner. In view of the foregoing amendments and remarks, Applicants respectfully request favorable reconsideration and early passage to issue of the present application.

Applicant's undersigned attorney may be reached in our Costa Mesa, California office at (714) 540-8700. All correspondence should continue to be directed to our below-listed address.

Respectfully submitted,

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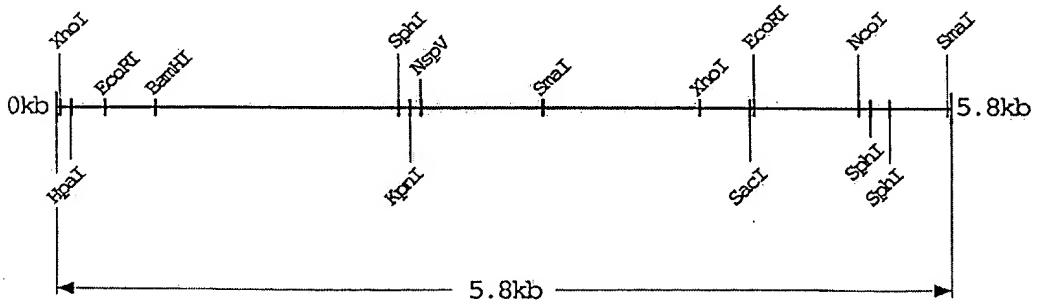
VERSION WITH MARKINGS TO SHOW CHANGES MADE TO CLAIMS

--1. (Twice Amended) An isolated DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene, having 1 BamHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, and having a restriction map of:



, said isolated DNA-fragment derived from [Borkholderia] Burkholderia cepacia KK01.

3. (Three Times Amended) An isolated DNA fragment having a nucleotide sequence that hybridizes under stringent conditions to a hybridization probe with a nucleotide

sequence consisting of SEQ ID NO: 1 or a complement of SEQ ID NO: 1[.] , said DNA fragment encoding a protein having a toluene monooxygenase activity.

5. (Twice Amended) The [recombinant DNA fragment] recombinant DNA according to Claim 4, wherein the vector can be maintained or replicated in a bacterium.

6. (Twice Amended) An isolated DNA fragment containing a region encoding a toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7 of the Sequence Listing, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form [an active monooxygenase] said toluene monooxygenase protein.

8. (Twice Amended) An isolated DNA fragment according to claim 6 or 7, further comprising a sequence encoding a polypeptide TomQ having an amino acid sequence of SEQ ID NO: 8 [in the Sequence Listing].

9. (Four Times Amended) An isolated DNA fragment containing a region encoding a toluene monooxygenase, wherein the region comprises a first sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 463..1455 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO:3 [or a mutant thereof], a second sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 1495..1761 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4 [or a mutant thereof], a third sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 1803..3350 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomN having an amino acid of SEQ ID NO: 5 [or a mutant thereof], a fourth sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 3428..3781 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6 [or a mutant thereof], and a fifth sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 3810..4871 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7 [or a mutant thereof], and the first to fifth sequences are aligned so that expressed polypeptides can form [an active monooxygenase] <u>said toluene monooxygenase</u> protein.

10. (Four Times amended) An isolated DNA fragment comprising a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide having a property of enhanced toluene monooxygenase activity.

13. (Amended) A recombinant DNA comprising [a vector; a first promoter and the DNA fragment encoding polypeptide TomK according to Claim 10 wherein the DNA fragment for TemK poly peptide is functionally linked to the first promoter to be expressed by the first promoter; a second promoter and the DNA fragment according to any one of claims 6 to 9 wherein the DNA fragment is functionally linked to the second promoter to be expressed by the second promoter] an expression vector comprising a first promoter and a first DNA fragment comprising a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide having a property ~~of~~ enhanced toluene monooxygenase activity, a second promoter and the DNA fragment according to any one of Claims 6, 7, and 9, wherein the first DNA fragment is functionally linked to the first promoter, and the second DNA fragment is functionally linked to the second promoter.

17. (Three Times Amended) A transformant obtained by introducing a recombinant DNA into a host microorganism, where the recombinant DNA comprises a vector enabling maintenance or replication in a host, said vector including a DNA fragment ligated

thereto having a sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of SEQ ID NO: 1 or a complement of SEQ ID NO: 1 and encoding an active toluene monooxygenase, wherein the DNA fragment [contains a toluene monooxygenase region of 4.9 kb or less] is 4.9 kb or less encoding a toluene monooxygenase.

19. (Amended) A transformant obtained by introducing a recombinant DNA comprising a vector, a promoter and a DNA fragment into a host microorganism where the DNA fragment contains a region encoding a toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form [an active monooxygenase] said toluene monooxygenase protein;

wherein the promoter and the DNA fragment are functionally linked enabling expression of the toluene monooxygenase protein encoded by the DNA fragment.

21. (Amended) A method for producing a toluene monooxygenase, comprising [a step of making the transformant according to any one of claims 15, 17 and 19

produce a toluene monooxygenase that is a gene product of the recombinant DNA introduced into the transformant.] the steps of:

culturing a transformant according to any one of claims 15, 17 and 19 in a medium; and

collecting the expressed toluene monooxygenase.

27. (Amended) The degradation method according to claim 22, wherein the aromatic compound is selected from the group consisting of [at least one of] toluene, benzene, phenol, and cresol.

33. (Amended) The cleaning method according to claim 28 wherein, the aromatic compound is selected from the group consisting of [at least one of] toluene, benzene, phenol, and cresol.

34. (Amended) A method for remedying an environment polluted with a pollutant being [at least either of] a chlorinated aliphatic hydrocarbon compound or an aromatic compound, comprising a step of degrading the pollutant by using the transformant according to any one of claims 15, 17 and 19.

48. (Amended) The remediation method according to claim 34, wherein the aromatic compound is selected from the group consisting of [at least one of] toluene, benzene, phenol, and cresol.

55. (Five Times Amended) A recombinant DNA comprising an expression vector comprising [a vector,] a promoter, a first DNA fragment and a second DNA fragment, said first DNA fragment being a DNA fragment of any one of claims 6, 7 or 9, said second DNA fragment being a DNA fragment comprising a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 234...443 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide having a property of enhanced toluene monooxygenase activity, [being the DNA fragment of any one of claims 6, 7 or 9, and a second DNA fragment, said second DNA fragment comprising a region encoding a polypeptide TomK having an amino acid sequence of SEQ ID NO: 2 and a property to enhance the toluene monooxygenase activity of a protein comprised of at least TomL to TomP; or a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 463..1455 portion of SEQ ID NO: 1 or a complement thereof, encoding TomK or an active mutant thereof,]

wherein the first DNA fragment containing a toluene monooxygenase encoding region of 4.9 kb or less is functionally connected to the promoter to express an active toluene monooxygenase, and the second DNA fragment is functionally connected to the promoter to express a property to enhance the toluene monooxygenase activity.--